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Fourier transform microwave spectroscopy of Ac-Ser-NH_2: the role of side chain interactions in peptide folding†

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Serine capped dipeptide *N*-acetyl-L-serinamide (Ac-Ser-NH_2) has been investigated using Fourier transform microwave spectroscopic techniques combined with laser ablation sources. Spectral signatures originating from one dominant species have been detected in the supersonic expansion. Rotational and nuclear quadrupole coupling constants of the two ^{14}N nuclei have been used in the characterization of a C_7^{eq}/γ-turn structure, which is stabilized by a CO···HN intramolecular hydrogen bond closing a seven-membered ring. Two extra hydrogen bonds involving the polar side chain ($-CH_2OH$) further stabilize the structure. The non-observation of C_5 species, attributed to the presence of the polar side chain, is in contrast with the previous gas phase observation of the related dipeptides containing glycine or alanine residues. The A–E splitting pattern arising from the internal rotation of the methyl group has been analyzed and the internal rotation barrier has been determined.

Introduction

Detailed knowledge of the mechanisms of protein folding is important in order to unravel structure–function relationships and improve our understanding of the numerous processes in living cell functions.[1] In the solution or the solid state, the protein folding process is a complex interplay of both intra- and intermolecular interactions with the solvent or surrounding amino acids of the protein. Although all these interactions might be present in their biological environment, in order to fully understand the intramolecular folding mechanisms it is also necessary to be able to investigate the intrinsic interactions within the proteins, in the absence of any solvent or crystal phase. In particular, non-covalent interactions such as intramolecular hydrogen bonds are interesting, since they define the present secondary structure. Gas phase experiments can provide conditions required for studying only the inherent properties of the molecules under investigation, free of interactions with the environment.[2,3]

The first step towards fully understanding protein folding requires detailed information on the intrinsic conformational preferences of amino acids, small peptides and peptide mimics. In particular, dipeptide mimics (containing two peptide linkages, –CO–NH–) have received much attention because they represent the smallest realistic and representative systems for designing local conformational effects in peptides and proteins. These molecules are also named capped dipeptides. The vast majority of this work has been done in molecular beam IR/UV double resonance experiments.[3,4] A disadvantage of those techniques, however, is that they require the molecule under study to possess a UV chromophore. Roughly speaking, this restricts this type of spectroscopy to molecules containing one or more aromatic rings, thereby excluding the majority of the amino acids and limiting strongly the different peptides that can be studied.

In contrast, Fourier transform microwave (FTMW) spectroscopy does not require any chromophore; it only needs the molecule to be studied to have a permanent dipole moment.[5] Therefore, it is particularly well suited for studying dipeptides containing amino acids as relevant as glycine, alanine or proline, elusive to IR/UV double resonance experiments. Lavrich *et al.*[6] investigated the alanine dipeptide *N*-acetyl-alanine *N*′-methylamide (Ac-Ala-NHMe) and observed only a C_7^{eq} conformation using heating methods to bring molecules into the gas phase. Very recently, the combination of FTMW spectroscopic techniques

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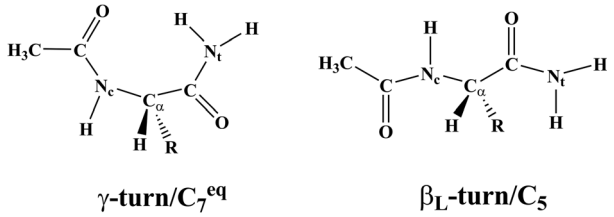
† Electronic supplementary information (ESI) available: Calculated spectroscopic parameters for conformers of Ac-Ser-NH_2 at the B3LYP/6-311++G(d,p) level of theory, measured frequencies for the nuclear quadrupole coupling hyperfine components and splittings for the A–E internal rotation components of the C_7^{eq}-I conformer of Ac-Ser-NH_2 together with the Cartesian coordinates for the *ab initio* predicted geometry of theory for the observed conformer of Ac-Ser-NH_2. See DOI: 10.1039/c5cp02654g



Scheme 1 Chemical structures of the C_7^{eq} (left) and C_5 (right) configurations for Ac-XX-NH_2 (XX = Gly and Ala) derivatives. N_c and N_t indicate central and terminal nitrogen atoms, respectively.

with laser ablation methods[7–10] has been successfully applied in the investigation of the conformational preferences of isolated protected dipeptides such as *N*-acetyl-glycinamide (Ac-Gly-NH_2),[11] *N*-acetyl-alaninamide (Ac-Ala-NH_2)[12] and *N*-acetyl-prolinamide (Ac-Pro-NH_2).[13] For both Ac-Gly-NH_2 and Ac-Ala-NH_2, the molecules were found to exist as both the C_7^{eq} (γ-turn) as well as the C_5 (β_L-turn) conformation (see Scheme 1), in which the backbones are stabilized by a CO···HN intramolecular hydrogen bond closing a seven- or five-membered ring, respectively. In contrast, in the investigation of the Ac-Pro-NH_2 only the C_7 (γ-turn) conformer was detected. The presence of the pyrrolidine ring provides rigidity to the peptide backbone and the formation of other configurations, possible in the other model peptides, is not observed for the Ac-Pro-NH_2 dipeptide.

The present work reports the first conformational study using Fourier transform microwave techniques of a dipeptide analogue containing an amino acid with a polar side chain, *N*-acetyl-L-serinamide (Ac-Ser-NH_2). The introduction of a polar side chain is an important step because amino acids with polar functional groups are very relevant to the protein function and structure.[14] In fact, serine residues have been found to have detrimental effects on the stability of transmembrane helices since they are involved in interhelical hydrogen bonds.[15] Moreover, the hydrogen bonds established by the polar group of serine residues have been shown to be at the origin of the activation processes of β_2-adrenergic receptors.[16,17] From the conformational point of view, the presence of a polar functional group, $-CH_2OH$, in the side chain of serine allows the establishment of additional intramolecular hydrogen bonds, which dramatically increase the number of low-energy conformers.[18] However, sometimes these extra intramolecular interactions are the motif of the selective stabilization of a determined conformation, as it has been shown for the natural α-amino acid asparagine.[19] These additional interactions do not occur in the aliphatic amino acids whose dipeptides have been previously studied. Consequently, the main goal of our research is to elucidate how this polar side chain of serine affects the conformational preferences of the dipeptide Ac-Ser-NH_2. Will a rich conformational space be observed? Or will the polar side chain favor one of the C_7^{eq} or C_5 conformations?

Experimental

A commercial sample of Ac-Ser-NH_2 (GeneCust, ~99%, m.p. ~183 °C) was used without any further purification. A solid rod was prepared by pressing the compound's fine powder mixed with a small amount of commercial binders and was placed in the ablation nozzle. A picosecond Nd:YAG laser (10 mJ per pulse, 20 ps pulse width) was used as a vaporization tool. Products of the laser ablation were supersonically expanded using the flow of carrier gas (Ne, 15 bar) and characterized by both chirped pulse Fourier transform microwave (CP-FTMW)[10] and laser ablation molecular beam Fourier transform microwave (LA-MB-FTMW)[20] spectroscopy. *N*-Acetyl-L-serinamide was first investigated using a CP-FTMW spectrometer to sample swiftly the rotational spectra of the different conformers present in the gas-phase mixture. Details of the experimental setup have been given elsewhere.[21] Chirped-pulses of 4 μs directly generated by the 24 Gs s^{-1} AWG were amplified to about 300 W peak power using a traveling wave tube amplifier. A parabolic reflector system composed of dual ridge horns and two parabolic reflectors in a paraxial beam configuration[21] was used to broadcast the excitation pulse and receive the broadband molecular emission. At a repetition rate of 2 Hz, a total of 70 000 free induction decays (4 FID emissions per gas pulse) each with 10 μs length duration were averaged and digitized using a 50 Gs s^{-1} digital oscilloscope. The frequency domain spectrum in the 6–12 GHz frequency range was obtained by taking a fast Fourier transform (FFT) following the application of a Kaiser–Bessel window to improve the baseline resolution.

The sub-Doppler resolution LA-MB-FTMW technique,[20] operating from 4 to 10 GHz, was used to record the rotational spectrum with the resolution necessary to analyze the hyperfine structure due to the presence of two ^{14}N nuclei in the molecule. A short microwave radiation pulse of 0.3 μs duration was applied to polarize all the vaporized molecules. The registered free induction decay was then converted to the frequency domain by Fourier transformation. All the transitions appeared as Doppler doublets due to the parallel configuration of the molecular beam and the microwave radiation. The resonance frequency was determined as the arithmetic mean of the two Doppler components. Frequency accuracy better than 3 kHz and an estimated resolution of 5 kHz are achieved in the experiment. From 50 to 100 averages were phase-coherently coadded to achieve reasonable signal to noise ratios (S/N).

Results and discussion

The recorded broadband rotational spectrum of laser ablated *N*-acetyl-L-serinamide from 6 to 12 GHz is shown in Fig. 1a. Decomposition product lines common to other studies of biomolecules[22,23] attributable to cyano-derivatives, water clusters, *etc.* were easily identified. After excluding the aforementioned signals from the spectral analysis, the identification of rotational transitions belonging to a single species was accomplished. Assignments were based on the identification of a-type $J+1_{0,J+1} \leftarrow J_{0,J}$ and $J+1_{1,J+1} \leftarrow J_{1,J}$ (with J ranging from 3 to 6) pairs of rotational progressions which appear to be splitted in two components as shown in Fig. 1b for 4_{14}–3_{13} and 4_{04}–3_{03} transitions. We attributed the splittings to the internal rotation of the methyl group attached to the N-terminal amide end of the observed rotamer.

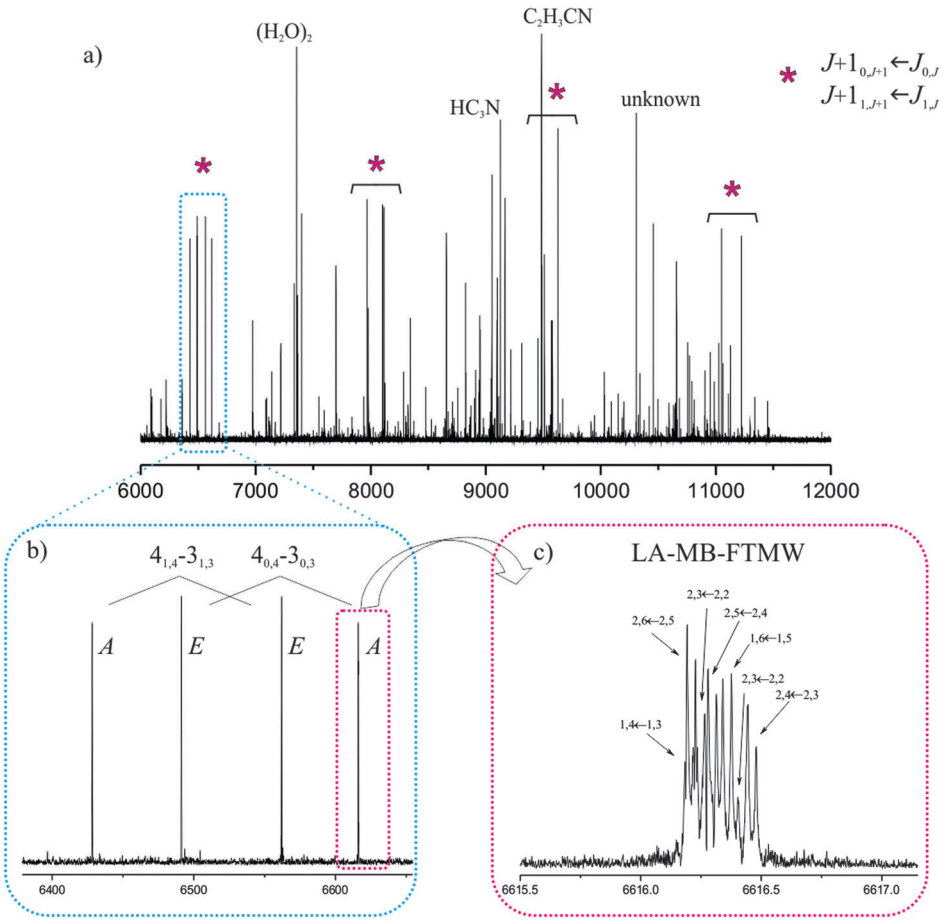


Fig. 1 (a) Broadband spectrum of Ac-Ser-NH_2 in the 6–12 GHz frequency region showing the most intense rotational transitions for the observed rotamer together with some of the photofragmentation product lines. (b) A section of the broadband spectrum showing the A–E states of the 4_{14}–3_{13} and 4_{04}–3_{03} rotational transitions. (c) The unperturbed A-state of the 4_{04}–3_{03} rotational transition of Ac-Ser-NH_2 showing its hyperfine structure completely resolved using a LA-MB-FTMW spectrometer. Each hyperfine component labeled with the corresponding values of I', $F' \leftarrow I''$, and F'' quantum numbers is split by the Doppler effect.

The V_3 torsional barriers for these methyl groups are low, as shown, for example, for the related molecule Ac-Ala-NH_2[12] causing the occurrence of the A–E splittings due to the coupling of the torsional vibration to the overall rotational angular momentum. Once the analysis of the μ_a-type spectrum was completed, μ_b-type transitions were subsequently predicted and measured with no μ_c-type spectrum being observed. Although several lines remained unassigned in the spectrum, the identification of further rotamers could not be achieved.

Both A and E components showed a partially resolved hyperfine structure corresponding to a compound with ^{14}N nuclei. This is because the ^{14}N nuclei have a non-zero quadrupole moment (I = 1) owing to a non-spherical distribution of the nuclear charge, which interacts with the electric field gradient created by the rest of the molecule at the site of these nuclei. The nuclear spin of ^{14}N nuclei couples to the rotational angular momentum resulting in a hyperfine structure in the rotational spectrum.[5] However, the spectral resolution attainable in the CP-FTMW experiments is not enough to completely resolve these hyperfine effects. For this reason, in a second stage of the investigation *N*-acetyl-L-serinamide was probed using our LA-MB-FTMW technique, which provides the high resolution needed to fully resolve this complicated hyperfine structure (as shown in Fig. 1c). Hence, a total of 89 hyperfine components from twelve ^aR- and three ^bR-branch transitions for the unperturbed A state (see Table S2 of the ESI†) were analyzed[24] using Watson's Hamiltonian $H = H_R + H_Q$, where H_R is the semirigid rotor Hamiltonian and H_Q describes the nuclear quadrupole coupling interaction.[25] The quadrupole coupling Hamiltonian was set up in the coupled basis set (I_1 I_2 I J F), $I_1 + I_2 = I$, and $I + J = F$.[26] The energy levels involved in each transition were thus labelled with the quantum numbers J, K_{-1}, K_{+1}, I, and F. The analysis rendered the accurate experimental rotational and nuclear quadrupole coupling constants shown in the left column of Table 1. The internal rotation barrier V_3 has been determined using the internal axis method in the form given by Woods.[27] The A–E splittings (see Table S3 in the ESI†) were fit using the program XIAM[28] yielding the internal rotation parameters summarized in Table 2.

In order to be able to identify the conformation corresponding to the observed rotamers in the spectrum, the conformational landscape of Ac-Ser-NH_2 was explored with the help of quantum

Table 1 Experimental and calculated spectroscopic parameters for the observed rotamer and the five lowest energy conformers of Ac-Ser-NH_2. *Ab initio* energies are included for the predicted species

	Experimental	C_7^{eq}-I[h]	C_5-I	C_7^{eq}-II	C_5-II	C_5-III
A[a]	1879.8183(73)[f]	1855	1957	2113	2017	1851
B	1001.544607(93)	1007	899	869	857	953
C	750.65138(13)	754	660	708	743	679
Δ_J	0.0573(25)	—	—	—	—	—
$\lvert\mu_a\rvert$	Y[g]	1.9	1.0	3.4	1.2	2.0
$\lvert\mu_b\rvert$	Y	0.4	0.3	0.7	0.3	1.8
$\lvert\mu_c\rvert$	N	0.1	0.5	2.2	0.2	0.3
N_c/χ_{aa}	1.9538(28)	2.00	2.38	2.16	2.25	2.19
N_c/χ_{bb}	−0.5099(41)	−0.43	0.86	−2.17	−1.04	1.20
N_c/χ_{cc}	−1.4439(41)	−1.56	−3.25	0.01	−1.21	−3.39
N_t/χ_{aa}	0.5880(31)	0.56	2.27	0.05	1.99	2.09
N_t/χ_{bb}	1.4018(48)	1.42	1.46	2.15	0.26	0.88
N_t/χ_{cc}	−1.9898(48)	−1.98	−3.73	−2.20	−2.22	−2.97
σ[b]	1.7	—	—	—	—	—
N[c]	89	—	—	—	—	—
ΔE[d]	—	0	1108	1221	1894	1617
ΔG[e]	—	0	659	892	1174	1187

[a] A, B, and C represent the rotational constants (in MHz); Δ_J is the quartic centrifugal distortion constant (in kHz), and χ_{aa}, χ_{bb} and χ_{cc} are the diagonal elements of the ^{14}N nuclear quadrupole coupling tensor (in MHz); N_c and N_t correspond to the central and terminal ^{14}N nuclei, respectively; μ_a, μ_b and μ_c are the electric dipole moment components (in D). [b] Rms deviation of the fit (in kHz). [c] Number of measured transitions. [d] Relative energies (in cm^{-1}) with respect to the global minimum calculated at the MP2/6-311++G(d,p) level of theory. [e] Gibbs energies (in cm^{-1}) calculated at 298 K at the MP2/6-311++G(d,p) level of theory. [f] Standard error in parentheses in units of the last digit. [g] Yes or No to observation of a-, b-, and c-type transitions. [h] The conformers are labeled by the size of the ring closed by the CO···NH hydrogen bond and an index going up with increasing energy.

Table 2 Methyl internal rotation experimental parameters for the identified rotamer of Ac-Ser-NH_2

V_3[a]	97.2 (3)[c] [127]
$<(i,a)$[b]	43.40 (2) [46]
$<(i,b)$	59.68 (2) [59]
$<(i,c)$	62.23 (2) [60]

[a] V_3 is the internal rotation barrier in cm^{-1}. [b] Experimentally determined values for the angles between the top rotational axis and the principal axis system, in degrees. In squared brackets, the theoretical values predicted for conformer C_7^{eq}-I are shown. [c] Standard error in parentheses in units of the last digit.

chemical calculations. In a first step, semiempirical calculations[29] were performed to search for all possible energetic minima of the Ac-Ser-NH_2 molecular system. The resulting molecular geometries were further optimized within the Gaussian suite of programs,[30] using a computationally effective B3LYP density functional model with Pople's 6-311++G(d,p) basis set and afterward by second-order Møller–Plesset (MP2) perturbation theory in the frozen core approximation with the same basis set. Frequency calculations were performed using both methods to compute the Gibbs free energies, which should be more representative of the relative populations of each structure. The derived rotational and ^{14}N nuclear quadrupole coupling constants together with the dipole moment components for the lowest-lying energy conformers (see Fig. 2) at the MP2/6-311++G(d,p) level of theory are shown in Table 1. This level of theory has been found to behave

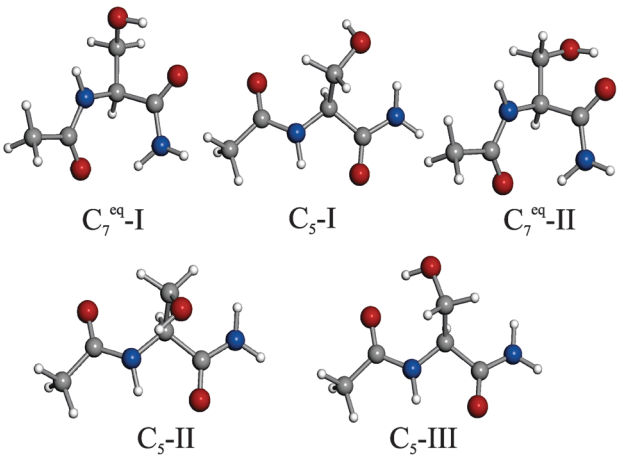


Fig. 2 The predicted five low-energy conformers of Ac-Ser-NH_2.

satisfactorily in previous studies of several biomolecules.[7–10,12,13] The results of the calculations at the B3LYP/6-311++G(d,p) level of theory are shown in Table S1 of the ESI.†

The conformational assignment of the observed rotamer was achieved by comparing the experimental spectroscopic constants with those predicted *ab initio*. The experimentally determined rotational constants are similar to those for the C_7^{eq}-I conformer. As we have recently shown,[11–13] nuclear quadrupole coupling constants can be used as fingerprints in conformational analysis of related dipeptides. These parameters are sensitive to the chemical environment of the nitrogen nuclei and to the orientation of the amino group with respect to the principal inertial axis system. Thus, a final comparison between the experimental and theoretical values for those constants clearly serves to discriminate between all the conformers and allows the unequivocal identification of the observed rotamer as conformer C_7^{eq}-I. The observed rotamer exhibited an intense μ_a-type spectrum and weak μ_b-type transitions, which is also in agreement with the identification of the C_7^{eq}-I conformer when considering the respective calculated values of the electric dipole moment components. Additionally, the methyl internal rotation experimental parameters are in good agreement with those estimated theoretically (Table 2) for conformer C_7^{eq}-I, which also supports the achieved assignment.

The experimental determination of the ^{14}N nuclear quadrupole coupling constants constitutes an exceptional tool that allows the unequivocal establishment of the orientation of the side chain $-NH_2$ and –NH groups with respect to the molecular frame. These constants can be used to deduce the nature of the intramolecular interactions in which this functional group is involved. Hence, in the C_7^{eq}-I conformer structure the acetyl carbonyl oxygen is hydrogen bonded to one of the terminal amide hydrogens (C═O···H–N_t), closing a seven-membered cycle in which the serine side chain is oriented equatorially. Moreover, the $-CH_2OH$ group of the serine side chain participates in two additional hydrogen bonds: one N_c–H···O–H and one O–H···O═C. As can be seen in Fig. 3, the estimated distances of the hydrogen bonds show that the O–H···O═C interaction is stronger than the N_c–H···O–H. This fact can be attributed to

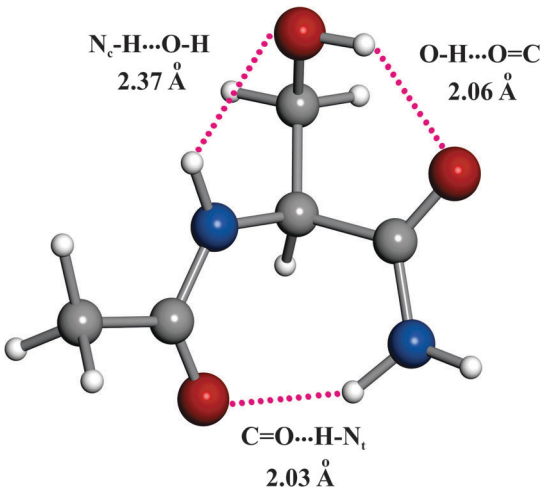


Fig. 3 3D *ab initio* structure (Cartesian coordinates in Table S4 of the ESI†) of the observed conformer of Ac-Ser-NH_2 showing the intramolecular interactions and the estimated distances which stabilize the structure.

the dominant donor character of the OH group over its acceptor propensity, leading to a quite unbalanced H-bonding network. The distance for the C═O···H–N_t bond (γ-turn) is analogous to those related for other γ-turns of aliphatic dipeptides such as Ac-Gly-NH_2[11] and Ac-Ala-NH_2,[12] for which intramolecular bond distances of 2.03 and 2.07 Å were found, respectively. This fact points to the fact that the intramolecular interactions of the side chain do not affect the strength of the γ-turn bond. However, the side chain extra interactions, which cannot take place in any other possible conformation of the Ac-Ser-NH_2, seem to be the factor which accounts for the overstabilization of this species and, thus, the non-observation of C_5 species. In contrast, for the related Ac-Gly-NH_2[11] and Ac-Ala-NH_2[12] dipeptides both C_7^{eq} and C_5 species were detected with the approximate population C_7^{eq}/C_5 ratio of around 2 and 3, respectively. Because no intramolecular interactions involving the lateral side chain can occur in Gly and Ala dipeptides, the stability/abundance difference between C_7^{eq} and C_5 species is exclusively determined by the strength of the C═O···H–N interactions (seven- or five-membered ring). On this basis, we can infer that the presence of polar side chains alters significantly the conformational preferences of dipeptides containing aliphatic amino acids.

The results obtained here for Ac-Ser-NH_2 can be compared with those reported previously for the analogous tripeptide Ac-Phe-Ser-NH_2 using IR-UV ion dip spectroscopy.[31,32] For the Ac-Phe-Ser-NH_2 molecule only the C_7/γ-turn conformer, where the C_7 ring established by the serine residue is structurally similar to that of the detected conformer of Ac-Ser-NH_2, was observed. Additionally, Yan *et al.*[31] found the same C_7 structure to be the most stable for the tripeptide Ac-Phe-Cys-NH_2. This peptide only differs by one atom with respect to Ac-Phe-Ser-NH_2, where the –OH side chain of serine is replaced by the –SH group in cysteine. For the Ac-Phe-Cys-NH_2 an additional structure which exhibits C_{10}/β-turn geometry was observed. The presence of the second structure was attributed to the weaker SH···O hydrogen bond strength, allowing the competition with other types of interactions such as SH–π interactions resulting in the presence of both γ-turn as β-turn conformations. This result confirms our conclusion that for Ac-Phe-Ser-NH_2, which exhibits a strong hydrogen bond, only one conformer is present, while when weaker H-bond interactions are formed such as for Cys or for the previously studied Gly[11] and Ala[12] capped peptides, other conformers besides this C_7 structure will be present.

Conclusions

The present investigation of the Ac-Ser-NH_2 system together with the previous work on Ac-Gly-NH_2, Ac-Ala-NH_2 and Ac-Pro-NH_2 illustrates the capabilities of the Fourier transform microwave techniques to investigate the conformational preferences of biologically relevant peptides isolated in the gas phase. The structural information derived for these aliphatic dipeptides, which are elusive to other high resolution spectroscopic techniques, is of utmost importance not only to gain knowledge about their intrinsic conformational properties but also to serve as a benchmark for theoretical investigations.

Our results for the serine dipeptide Ac-Ser-NH_2 show that its conformational landscape in the gas phase is dominated by a single C_7^{eq}/γ-turn species. Now, the initial research question about whether the weaker polar side chain favors one of the C_7 and C_5 conformations through the formation of intramolecular hydrogen bonds can be answered. The additional intramolecular interactions formed by the presence of a polar group in the side chain of serine have been shown to be at the origin of the conformational locking to a C_7^{eq} species observed for the dipeptide Ac-Ser-NH_2. Hence, it has been demonstrated that the presence of a polar side chain increases the plausible number of conformations but in contrast imparts restrictions on the amount of conformers observed.

Acknowledgements

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